

## The rate-limiting step for glucose transport into the hypothalamus is across the blood–hypothalamus interface

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### Abstract

Specialized glucosensing neurons are present in the hypothalamus, some of which neighbor the median eminence, where the blood–brain barrier has been reported leaky. A leaky blood–brain barrier implies high tissue glucose levels and obviates a role for endothelial glucose transporters in the control of hypothalamic glucose concentration, important in understanding the mechanisms of glucose sensing. We therefore addressed the question of blood–brain barrier integrity at the hypothalamus for glucose transport by examining the brain tissue-to-plasma glucose ratio in the hypothalamus relative to other brain regions. We also examined glycogenolysis in hypothalamus because its occurrence is unlikely in the potential absence of a hypothalamus–blood interface. Across all regions the concentration of glucose was

comparable at a given plasma glucose concentration and was a near linear function of plasma glucose. At steady-state, hypothalamic glucose concentration was similar to the extracellular hypothalamic glucose concentration reported by others. Hypothalamic glycogen fell at a rate of  $\sim 1.5 \mu\text{mol/g/h}$  and remained present in substantial amounts. We conclude for the hypothalamus, a putative primary site of brain glucose sensing that: the rate-limiting step for glucose transport into brain cells is at the blood–hypothalamus interface, and that glycogenolysis is consistent with a substantial blood-to-intracellular glucose concentration gradient.

**Keywords:** blood–brain barrier, glucose transport, glycogen, hypothalamus.

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The brain is remarkable in its dependence on the blood for essentially two energy substrates, i.e., glucose and oxygen. To maintain the brain's high rate of aerobic metabolism, transport of glucose into the brain occurs through regionally selective, bidirectional, facilitated transport (Lund-Andersen 1979; Pardridge 1983; Bradbury 1993). The brain microenvironment is isolated from the plasma by the blood–brain barrier (BBB) formed by tight junctions between vascular endothelial cells, where tightness excludes free diffusion of polar, hydrophilic molecules such as glucose resulting in a constant and significant glucose concentration gradient from blood to brain, i.e., approximately 5–1 [(Lund-Andersen 1979) and references therein; (Gruetter *et al.* 1998) and references therein; (Barros *et al.* 2007)]. Glucose is transported into brain by the major high affinity glucose transporter isoform GLUT1 (Pardridge *et al.* 1990), distributed between the luminal and abluminal membranes of the capillary endothelial cells that comprise the BBB *in vivo* (Carruthers 1990).

Increases of both GLUT1 synthesis and luminal GLUT1 mediate enhanced glucose transport across the BBB into rat brain in hypoglycemia (Simpson *et al.* 2007). The integrity of the BBB for glucose transport is therefore expected to profoundly impact the concentration of glucose in the brain microenvironment [Gruetter *et al.* (1998) and references therein] and in brain cells that use glucose either as a signaling molecule or energy substrate for phosphorylation

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*Abbreviations used:* BBB, blood–brain barrier; CSF, cerebral spinal fluid

and/or whose glucosensing is altered in hyper/hypoglycemia (Mobbs *et al.* 2001; Levin *et al.* 2004; Marty *et al.* 2005; Song and Routh 2005, 2006). This is particularly relevant to the hypothalamus: two hypothalamic glucose-sensing structures, the arcuate nucleus and the ventral medial nucleus neighbor the hypothalamic median eminence, a circumventricular organ that lacks a strict BBB (Fry and Ferguson 2007). It has therefore been suggested that the BBB at the hypothalamus is absent or leaky (Cheunsuang *et al.* 2006; Mountjoy and Rutter 2007). The potential absence of a diffusion barrier for glucose is expected to lead to elevated hypothalamic glucose concentrations relative to that in other brain regions. The question remains as to what the concentration of glucose in the hypothalamus is since it is a key parameter linking glucose transport and metabolism (Gruetter *et al.* 1998; Barros *et al.* 2007), and whether the brain tissue-to-plasma glucose ratio in the hypothalamus is similar to other brain regions that have intact barriers.

When plasma glucose levels reach approximately 2 mM in hypoglycemia, decreases in cerebral metabolic rates of glucose ( $\text{CMR}_{\text{glc}}$ ) have been reported to exceed those of oxygen in the conscious rat (Ghajar *et al.* 1982) indicating that the brain uses other energy substrates, one of which is glycogen. Brain glycogen, a predominantly glial store of glucose equivalents, is an important endogenous energy source to maintain brain cell function when blood–brain glucose transport becomes rate-limiting during acute hypoglycemia (Choi *et al.* 2003; Gruetter 2003). Brain glycogen levels are sensitive to changes in tissue and plasma glucose concentrations (Choi *et al.* 2003; Morgenthaler *et al.* 2006) and replete to levels higher than pre-depletion levels in brain following a single episode of hypoglycemia (Choi *et al.* 2003; Oz *et al.* 2003).

This rebound in glycogen, known to be neuroprotective through the breakdown into mostly glucose-6-P which is glycolyzed to pyruvate and which has been suggested to support neuronal metabolism in hypoglycemia, most likely in the form of lactate (Gruetter 2003), may affect glucose sensing by specialized neurons as they also respond to lactate (Song and Routh 2005), and this may in part explain loss of physiological counter-regulatory defense mechanisms in insulin-induced hypoglycemia in diabetic patients undergoing intensive insulin therapy (Bolli 2003; Criego *et al.* 2005).

Strong evidence suggests that the hypothalamus is a key region in the brain responsible for hypoglycemia sensing and for triggering physiological counter-regulatory defense mechanisms (Borg *et al.* 1997) and the question remains as to how glycogen metabolism is affected in the putative primary site of glucose sensing, the hypothalamus. The potential absence of a BBB at the hypothalamus is expected to lead to similar concentrations of blood and brain glucose, i.e., glucose phosphorylation will only be limited when plasma glucose approaches the  $K_m$  of hexokinase (50  $\mu\text{M}$ ),

which is practically impossible and thus glycogenolysis would be unlikely.

The present work extends our pioneering NMR spectroscopy data of the relationship between glucose concentration in blood and in the brain by (i) improving the spatial resolution of the measurements; and (ii) by validating previous NMR measurements of glucose and glycogen in brain tissue by a biochemical method.

Therefore, the purpose of this study was: (i) to assess the integrity of the hypothalamic–blood interface relative to other brain regions for the transport of glucose and (ii) to examine whether, in support of a functional hypothalamic–blood interface, the hypothalamus exhibits glycogen breakdown. A preliminary report of part of this work has appeared (Poitry-Yamate *et al.* 2007).

## Materials and methods

### Animal preparation

The study was performed in accordance with Swiss Federal guidelines for the care and use of laboratory animals and was approved by the ethics committee. Male Sprague-Dawley rats (Charles River, France) weighing between 250–280 g were fasted overnight with access to water. To enable comparison between biochemical and previous *in vivo* NMR studies, measurements in the present study were obtained from hypoglycemic, euglycemic and hyperglycemic rats following the same preparatory glycemic procedures carried out for *in vivo*  $^1\text{H}$  and  $^{13}\text{C}$  NMR experiments (Choi *et al.* 2001). Briefly, three groups of animals were studied at target plasma glucose levels: hypoglycemia (<2 mM), euglycemia (5–10 mM) and hyperglycemia (>10 mM). All animals were initially anesthetized with 4% isoflurane in a 2 : 1 mixture of nitrous oxide and oxygen during induction. Isoflurane was lowered to 2% during catheterization. Animals were artificially ventilated with a pressure-driven ventilator. For hyperglycemia and hypoglycemia studies,  $\alpha$ -chloralose was given as an 80 mg/kg bolus followed 10 min later by infusion at a rate of 27 mg/kg/h. Hyperglycemic animals were maintained at a level of  $\sim 17$  mM plasma glucose by adjustment of glucose infusion rate.

Insulin (1 U/mL Humulin®; Lilly Inc, Paris, France) was administered to reach a target hypoglycemia condition of plasma glucose <2 mM for 2–3 h. The duration of hypoglycemia and hyperglycemia before blood sampling and brain fixation was on average 3 and 7 h, respectively. Because no significant difference was found for total brain glucose and glycogen as a function of plasma glucose between the light anesthetics isoflurane and  $\alpha$ -chloralose (Morgenthaler *et al.* 2006), we elected to use only isoflurane anesthesia in the euglycemic studies. Plasma glucose in euglycemic animals was maintained between 5 and 10 mM by adjustment of glucose infusion rate.

### Microwave fixation and brain dissection

After final blood sampling, anesthetized animals underwent focused microwave irradiation of 4 kW at 2450 MHz for 2 s (Gerling Applied Engineering Inc., Modesto, CA, USA). 2 s was sufficient to provide absorption of microwave energy (and hence heat generation

and subsequent temperature rise) between superficial and deep brain regions. Decapitation was performed after microwave fixation. We have established that this approach leads to minimal agonal metabolism as verified from the tissue lactate levels (Morgenthaler *et al.* 2006). The cortical surface of the brain was then exposed, meninges removed and five brain regions of interest were dissected within 2 min in the following order: olfactory bulb, hypothalamus, then coronal sections of frontal cortex, hippocampus and cerebellum. Care was taken to consistently take the same region in coronal sections and to keep tissue sample volumes similar, i.e.  $<1 \times 3 \times 3 \text{ mm}^3$  or  $\sim$  between 5–10 mg of wet weight tissue.

### Biochemical measurements

Following the determination of wet weight of each freshly dissected brain sample, the sample was immediately immersed in 250  $\mu\text{L}$  of 0.03 M HCl, the contents were homogenized using an ultrasonic homogenizer probe microtip (Bandelin, Berlin, Germany), and immediately divided into two equal volumes, one for determination of free glucose and the second for the determination of digested glycogen plus free glucose.

The release of glucose from glycogen by amyloglucosidase (10 mg/mL, Roche Applied Science, Rotkreuz, Switzerland) was performed following the procedure described by Cruz and Dienel (Cruz and Dienel 2002). Samples digested by amyloglucosidase from tissue at euglycemia and hyperglycemia were diluted between two and five-fold in water (pH 7.4) such that spectroscopic absorbance values fell in the middle of the glucose-absorbance calibration curve.

Tissue glucose, whether for the background or digested sample, was assayed using Trinder's method (Amplex Red, Invitrogen, Basel, Switzerland) and the samples in the present study were not frozen prior to glucose measurements. Glucose Standards, prepared from commercially available D-glucose, were left at room temperature (22°C) for  $\sim 3$  h prior to use. This assay is based on glucose oxidase digestion, forming  $\text{H}_2\text{O}_2$  which reacts with peroxidase substrate in the presence of horseradish peroxidase to generate resorufin. All samples, including standards, were brought to pH 7.4 using NaOH and equal volumes of samples and reaction buffer reagent, typically 60  $\mu\text{L}$ , were used. 100  $\mu\text{L}$  of the final 120  $\mu\text{L}$  solution of (sample + reaction buffer reagent) was transferred under dim room light to a 96-well microplate and left in darkness for 30 min at room temperature before absorption measurements. Maximum absorbance was attained at room temperature at  $\lambda = 560 \text{ nm}$ , pH  $> 7$ , 30 min after the reagent was added. Absorbance as a function of D-glucose concentration was measured using a Biotek Powerwave XS spectrophotometer (Bio-Tek Instruments, Inc. Burlington, VT, USA).

Absorbance values of brain glucose and brain glycogen, i.e., the difference between digested and background glucose) were expressed in units of  $\mu\text{mol/g}$  wet weight using the equation:

$$[\text{Glc}] = \frac{(V_{\text{ab}} - A)/B}{H}, \quad (1)$$

where  $V_{\text{ab}}$  is the absorbance value,  $A$  is the  $y$ -intercept; and  $B$  is the slope from linear regression plots of absorbance as a function of glucose concentration.  $H$  is the starting dilution factor = (mg tissue wet weight)/(250  $\mu\text{L}$  0.03 M HCl + mg tissue wet weight).

### Determination of glucose transport kinetics

To allow comparison with previous whole-brain rat NMR results, i.e., under similar experimental conditions and anesthesia protocols, we measured brain glucose as a function of plasma glucose at steady-state and express the relationship as a linear function and report the apparent kinetic constants (Gruetter *et al.* 1998; Pfeuffer *et al.* 2000; Choi *et al.* 2001; Lei and Gruetter 2006) from the linear regression plots of plasma glucose and tissue glucose using the expression:

$$G_{\text{brain}} = V_d \frac{\left( \frac{T_{\text{max}}}{\text{CMR}_{\text{Glc}}} - 1 \right) G_{\text{plasma}} - K_t}{\frac{T_{\text{max}}}{\text{CMR}_{\text{Glc}}} + 1}. \quad (2)$$

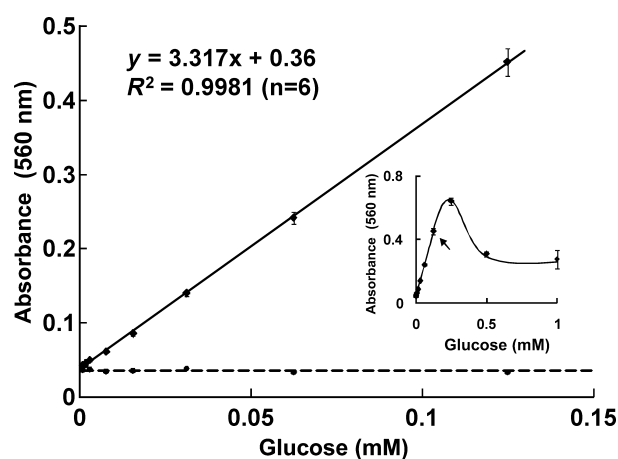
Where  $G$  is the concentration of glucose by brain region indicated ( $\mu\text{mol/g}$ ) or in plasma ( $\text{mmol/L}$ );  $V_d = 0.77 \text{ mL/g}$ , the physical distribution space of water in brain;  $T_{\text{max}}$  is the apparent maximum transport rate,  $\text{CMR}_{\text{glc}}$  is the cerebral metabolic rate of glucose utilization and  $K_t$ , the apparent Michaelis–Menten constant. The equation was fitted using OriginPro 7.5 (OriginLab Corporation, Northampton, MA, USA).

### Results

To measure glucose and/or glycogen from small volumes (5–10  $\mu\text{L}$ ) of tissue samples, we implemented an enzymatic-absorbance based assay previously used in quantifying hepatic glycogen (Dong *et al.* 2006) and plasma glucose (Koch and Nipper 1977). This assay was inherently sensitive, and between 0.00095 and  $\sim 0.125 \text{ mM}$  glucose, with a precision of  $< 2\%$  (Fig. 1). Omission of the Amplex Red reagent (shown by the dotted line) to verify reaction specificity resulted in constant absorbance.

Tissue glucose concentrations were comparable at a given plasma glucose concentration across all brain regions, averaging 1.6  $\mu\text{mol/g}$  at euglycemia (5–10 mM plasma glucose), 0.2  $\mu\text{mol/g}$  at hypoglycemia ( $< 2 \text{ mM}$  plasma glucose) and 4.0  $\mu\text{mol/g}$  at hyperglycemia (10–22 mM plasma glucose). In all five brain regions the concentration of tissue glucose consistently increased in a nearly linear fashion with plasma glucose concentration (Fig. 2). Of particular interest was the 5 : 1 ratio observed at euglycemia between glucose concentration in blood and hypothalamus (Fig. 2). The brain/plasma glucose concentration ratio indicated for each brain region in Fig. 2 was similar in the various brain structures and not statistically significant from each other.

The relationship between brain and plasma glucose concentration was fit using Eqn (2). Values of  $T_{\text{max}}/\text{CMR}_{\text{glc}}$  varied from  $2.5 \pm 0.2$  (hypothalamus and cortex),  $1.9 \pm 0.2$  (cerebellum).  $K_t$  was on the order of a few mM:



**Fig. 1** Sensitivity, linearity and reproducibility of glucose assay using glucose oxidase. Standards of D-glucose ranging from 0.00095 to ~0.125 mM were linearly related to absorbance of resorufin at 560 nm in the presence of reagent ( $n = 6$  replicates). The dotted line corresponds to the fit of the same glucose standards in the absence of reagent ( $n = 2$  replicates). Inset shows regions of non-linearity of assay, starting from arrow.

$3.2 \pm 0.9$  mM in hippocampus and cortex;  $1.5 \pm 0.9$  mM in olfactory bulb,  $0.1 \pm 1.0$  mM in cerebellum and  $2.6 \pm 1.1$  mM in hypothalamus.

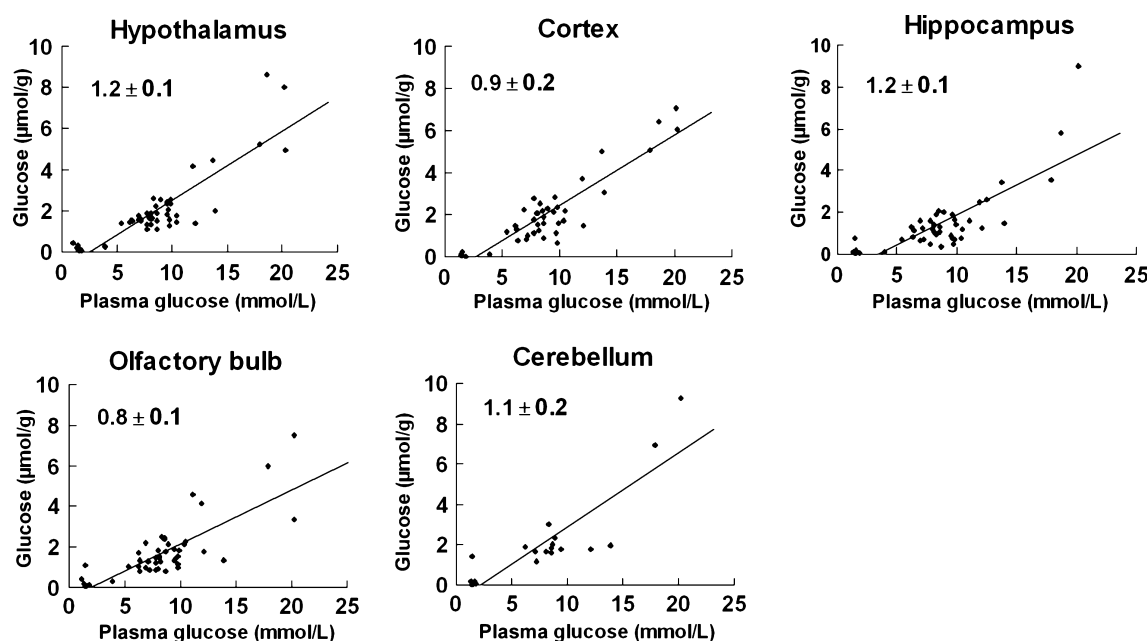
The effect of glycemia on glycogen concentration and metabolism in the hypothalamus is shown in Table 1 and

**Table 1** Glycogen content by brain region and plasma glucose concentration

	Hypoglycemia <2 mM	Euglycemia 5–8 mM	Hyperglycemia >10 mM
Hypothalamus	$1.9 \pm 0.8$ (8)**	$4.4 \pm 1.0$ (14)	$5.5 \pm 1.6$ (7)
Cortex	$0.9 \pm 1.2$ (5)*	$4.0 \pm 1.4$ (13)	$5.1 \pm 1.9$ (9)
Olfactory bulb	$1.7 \pm 1.0$ (8)	$2.9 \pm 0.8$ (16)	$5.1 \pm 1.4$ (8)**
Hippocampus	$1.8 \pm 1.5$ (7)	$3.4 \pm 1.1$ (13)	$3.4 \pm 1.9$ (8)
Cerebellum	$1.5 \pm 1.3$ (5)*	$4.4 \pm 1.6$ (5)	$6.1 \pm 1.9$ (4)

Values are mean  $\mu\text{mol/g} \pm \text{SD}$  based on ( $n$  measurements). Glycogen content in hypoglycemia and hyperglycemia was compared with that in euglycemia using two-way ANOVA following the Bonferroni post-tests. \*Indicates a significant difference for  $p < 0.01$  and \*\* for  $p < 0.001$ .

compared with four other brain regions. After 2 h of hypoglycemia ( $< 2$  mM plasma) glycogen remained present in substantial amounts in all five brain regions. Glycogen was reduced from  $\sim 4.5$  to  $\sim 1.6$   $\mu\text{mol/g}$ , equivalent to a rate of  $\sim 0.025$   $\mu\text{mol/g/min}$  (for hypothalamus, cortex and cerebellum) and from  $\sim 3.2$  to  $\sim 1.8$ , equivalent to a rate of  $\sim 0.011$   $\mu\text{mol/g/min}$  (for olfactory bulb and hippocampus). When plasma glucose was raised to  $>10$  mM from euglycemia, glycogen content increased slightly in all five regions and reached statistical significance in olfactory bulb ( $p < 0.001$ , Table 1).



**Fig. 2** Relationship between brain and plasma glucose concentration from anatomically and functionally distinct brain regions. Regression lines of plots show the best-fit of the model of glucose transport (as described in Materials and methods) to the data points measured biochemically over an extended range of plasma glucose levels

( $\sim 1.4$  to  $22$  mM). All brain regions showed near linearity. The mean values of the slope  $\pm$  SD by brain region are indicated. Linear regression plots were then used to determine the kinetic parameters,  $K_t$  and  $T_{\text{max}}/\text{CMR}_{\text{glc}}$ .  $> 40$  independent glucose measurements were performed per brain region except for cerebellum ( $n = 19$ ).



Levels of tissue glycogen and glucose as a function of plasma glucose for the hypothalamus and cortex are given in Table 1 and Fig. 2, and show that in both regions glycogen exceeds glucose levels.

## Discussion

We implemented a highly sensitive and reproducible enzyme and absorbance-based assay using glucose oxidase (Fig. 1). In conjunction with uniform microwave fixation of superficial and deep brain regions, the enzymatic assay allowed the use of small, 10  $\mu$ L volumes of tissue with minimal partial volume effects. It is important to note that beyond 125  $\mu$ M glucose, the absorption of resorufin was no longer linear (Fig. 1, inset), necessitating care to choose appropriate sample dilutions. We went on to use this biochemical assay to determine whether the brain tissue-to-plasma glucose ratio was similar in the hypothalamus compared with other brain regions of  $\alpha$ -chloralose anesthetized rats, and also at hypoglycemia and hyperglycemia.

The linearity shown in Fig. 2 by brain region was in excellent agreement with that previously measured for whole brain and lends support to the notion that the whole brain represents the weighted average of the levels of its component parts (Gruetter *et al.* 1996, 1998; Choi *et al.* 2001, 2002; de Graaf *et al.* 2001; Lei and Gruetter 2006).

The similar brain tissue-to-plasma glucose ratio between hypothalamus and the other anatomically distinct brain regions (Fig. 2) suggests similar glucose transport kinetics. Implicit in all aforementioned glucose transport models is the assumption that: (i) the concentration gradient of glucose is primarily at the entry of glucose into the brain, i.e., the endothelial cells or astrocytic endfoot processes, and (ii) that glucose is uniformly distributed in the brain aqueous phase beyond the BBB. Indeed, a  $\sim 5 : 1$  ratio of plasma to tissue glucose concentration at euglycemia across all brain regions, including the hypothalamus (Fig. 2) supports the presence of a significant glucose concentration gradient from blood to brain (Gruetter *et al.* 1992, 1996, 1998). Total tissue glucose concentrations, which represent extracellular and intracellular glucose levels, have been indirectly assessed from the equilibrium brain/plasma distribution ratio of 3-O-methyl-D-glucose (Gjedde and Diemer 1983; Namba *et al.* 1987; Dienel *et al.* 1991; Nakanishi *et al.* 1996) implying a relative regional uniformity of brain glucose concentrations. However, 3-O-methylglucose differences between the extracellular space and intracellular space have not been established.

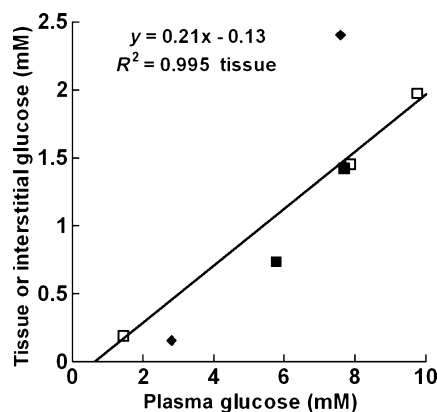
We have previously shown that deep anesthesia increases brain glucose content at a given plasma glucose concentration (Choi *et al.* 2002) and the anesthesia in the present study might have led to an overestimated hypothalamic glucose concentration. However, the presence of a significant blood/brain glucose concentration gradient in anesthetized brain

only underlines the presence of a significant diffusion barrier for glucose entry into the hypothalamus.

When comparing the tissue glucose measurements with extracellular, i.e., interstitial glucose measurements in hypothalamus (Fig. 3) the two were on the same order of magnitude and clearly lower than the plasma glucose concentration, with the implication that at steady-state, glucose concentrations are similar in the intracellular and extracellular space. This observation strongly suggests a structural and functional transport barrier for glucose entry across the hypothalamus–blood interface. Even if deeper anesthesia was used in some of the extracellular measurements used for Fig. 3, the main conclusion is unaffected by the specific degree of anesthesia, since deeper anesthesia tends to reduce the concentration gradient (Choi *et al.* 2002).

In addition to the BBB, other potential routes of entry of glucose into the hypothalamus are indirectly via the CSF (Broadwell *et al.* 1983) or via transport-independent mechanisms (Faouzi *et al.* 2007). A significant CSF-to-median eminence exchange that would elevate the concentration of glucose preferentially in hypothalamus is, however, unlikely: immunohistochemical evidence shows low to absent expression of GLUT1 in hypothalamic  $\beta$ 2-tanycytes that participate in forming an efficient barrier system between the median eminence and CSF (Peruzzo *et al.* 2000; Garcia *et al.* 2001) and influx is expected to be minimal because of a  $10^3$ -fold lower circumventricular organ surface area relative to that of the BBB (Kastin and Pan 2006).

The relationship established between increased whole brain glucose concentration and reported changes of endo-



**Fig. 3** Comparison of tissue and interstitial glucose concentration for hypothalamus as a function of plasma glucose. The concentrations of hypothalamic glucose (open squares) were those determined in the present study (Fig. 2) at plasma glucose concentrations (in mM) of  $1.5 \pm 0.03$ ;  $7.9 \pm 0.1$ ;  $9.8 \pm 0.1$ ;  $n = 5$  per point. Interstitial glucose concentrations: zero-net flux in unrestrained rats (closed squares) from (de Vries *et al.* 2003); and implanted glucose microelectrodes in pentobarbital-anesthetized rats (closed triangles) from (Silver and Erecinska 1994).  $R^2$  refers to hypothalamic tissue glucose.

thelial GLUT1 protein in sustained hypoglycemia suggests that a normal glucose environment in brain can be created as a consequence of increased GLUT1 expression and up-regulation and/or sequestration of GLUT1 protein at the BBB mediated by ambient glucose concentration (Lei and Gruetter 2006). Such a mechanism is a likely alternative explanation to the observed hypoglycemia unawareness in patients undergoing intensive insulin treatment of diabetes, although sequestration of GLUT1 at the BBB might also allow for acute regulation of glucose transport (Barros *et al.* 2005). The similar values obtained of kinetic constants for glucose transport at steady-state between whole brain measurements and anatomically and functionally distinct brain regions suggests that such a blunting of normal glucose sensing by the brain may specifically involve increased GLUT1 expression and up-regulation and/or sequestration of GLUT1 protein at the BBB at the hypothalamus, a key glucose sensing region for triggering hypoglycemic counter-regulatory defense mechanisms (Borg *et al.* 1997).

Under euglycemic conditions, the concentration of glycogen in all five regions of the brain was higher than that of the corresponding brain glucose (cf. Fig. 2 and Table 1) suggesting that the brain synthesizes and stores glycogen as a likely reserve supply of energy (Gruetter 2003). Indeed, the finding that 2 h of severe, acute hypoglycemia did not completely deplete brain glycogen in any brain region studied (Table 1) demonstrates that functionally distinct areas of the brain may survive extended periods of hypoglycemia through glycogenolysis. These observations extend the importance of glycogen as being neuroprotective when BBB glucose transport becomes rate-limiting for glucose metabolism (Choi *et al.* 2003; Lei and Gruetter 2006; Morgenthaler *et al.* 2006; Suh *et al.* 2007). At a plasma glucose concentration of 1.5 mM attained during hypoglycemia in the present study, we estimated a 10% glucose deficit in hypothalamus, cortex and hippocampus. Therefore, glycogenolysis accounts for a substantial fraction of the estimated 10% glucose supply deficit for all reported and estimated glucose metabolic rates (Sokoloff *et al.* 1977), as previously reported for whole rat brain (Choi *et al.* 2003).

Similar net glycogen utilization rates in cortex and hippocampus have been reported in unrestrained rats (Herzog *et al.* 2008), after taking into account differences in the severity and duration of hypoglycemia (1.9 vs 1.5 mM; and 3 vs. 2 h). However, in that study, the reported glycogenolytic rate was 50% lower than ours in the hypothalamus and this difference cannot be explained by differences in the measured, control glycogen concentrations, which were similar.

In previous brain studies, hyperglycemia was shown to increase glycogen content (Nelson *et al.* 1968; Swanson *et al.* 1989; Morgenthaler *et al.* 2006). Here, a similar trend was observed (Table 1) but not statistically significant for all brain regions. Future studies are needed to establish glycogen

levels and brain tissue-to-plasma glucose ratios by brain region in hyperglycemia, which would suggest that tissue glucose itself may affect glycogen levels.

In conclusion, biochemical evidence has been presented in support of a functional hypothalamus–blood interface for the transport of glucose. We speculate that in such an environment, specialized glucosensing neurons in the hypothalamus may effectively monitor and respond to the availability of glucose [for reviews, see Routh *et al.* (2004); Marty *et al.* (2007); McCrimmon (2008)]. In this connection, and beyond the BBB, accumulating evidence shows that neuron function is finely modulated by surrounding glia, such that these two major cell types interact synergistically (Poitry-Yamate *et al.* 1995), inviting the question as to the extent the process of glucose sensing is altered by the function of glial cells and glial glycogen.

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## Conflicts of interest

All authors declare no conflicts of interests.

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